Protocols for chemicals

10X TBE buffer, for gels electrophoresis

- Dissolve 108g of Tris base, 55g of Boric Acid and 7.5g of EDTA, disodium salt into approximately 800mL of ddH_2O. Use a magnetic stirrer to stir until the solution gets clear;
- Dilute the mixture into 1L.
- Autoclave;

1X TM buffer (40 mM Tris, 100mM MgCl2, pH 8), for nanostructure assembly

Volume to be prepared: 50ml (can prepared less if it is possible to weigh less MgCl2)
Materials needed: 0.566055g MgCl2 · 2H2O or 0.476055g MgCl2 or **1.0165g MgCl2.6H2O**

- 2ml 1M Tris solution (prepared before) + 48ml ddH2O to make 40mM Tris solution
- Dissolve 0.566055g MgCl2 · 2H2O (or 0.476055g MgCl2) into the solution
- Adjust pH to pH 8 by adding HCl

- Dilute to 1X by adding 115µL 2X TM buffer + 115µL ddH2O to an eppendorf, then mix
- Autoclave

Triton-X 100 Buffer, for ABTS Assay/Gq formation

50 mM Tris–HCl, 150 mM NH4Cl, 20 mM KCl, and 0.03% Triton X-100, pH 7.5
Volume to be prepared: 100ml

NH4Cl: 0.802365g
KCl: 0.1491026g
Triton X-100: 30ul
Adjust pH by adding HCl

Preparation

1. 5ml 1M Tris + 95ml ddH2O
2. Dissolve 0.8g NH4Cl, 0.15g KCl
3. Adjust pH by adding HCl

4. Add 30ul Triton X-100

GelDye(1000uL), for gel loading

- 100X Gel Red Stain 60uL
- 100% Glycerol 600uL
- ddH_2O 340uL

NB for mixing with samples, add at a sample-Dye ratio of 5:1

LB Agar (Antibiotic resistance: Chloramphenicol), for bacterial growth media

- Weigh 10g NaCl, 10g Tryptone, 5g Yeast Extract and 15g Agar;

- Mix thoroughly the above with 1L of ddH_2O;
- Autoclave at 121°C for 15minutes;
- Let the agar to cool to 55°C;
- Add at a concentration 25ug/mL (25mg in total for 1L) of chloramphenicol to the cooled agar;
- Aseptically,.pour ~20mL LB agar per 10cm polystyrene Petri dish for the plates to growth *E. coli* DH10B;
- Cover with lid and allow the plates to cool for 30-60minutes at room temperature, or until set.
- Label bottom of plates with antibiotic resistance 'CmR' and store in plastic bags at 4°C.
- For those with colonies, seal them with parafilm and store them separately at 4°C.

LB Broth, for Mini-prep

- Weigh 10g Bacto-tryptone, 5g yeast extract and 10g NaCl;
- Mix thoroughly the above with 800mL of ddH_2O;
- Adjust pH to 7.5 with NaOH;
- Adjust volume to 1L with ddH_2O;
- Sterilize by autoclaving;

SOB Media (and SOC media), for cell recovery after transformation

- Weigh 20.0g Tryptone, 5.0g Yeast Extract, 0.5 Sodium Chloride, 2.4 anhydrous Magnesium Sulphate and 186 mg Potassium Chloride;
- Mix thoroughly the above with 1L of ddH_2O;
- Autoclave at 121°C for 15minutes;

- For **SOC Media**, add 20mL filter-sterilized 20% glucose solution after cooling the SOB medium to 45-50°C max. Do this aseptically. Shake to mix.

Agarose Gel

- Weigh, according to the % of the gel required, the amount of agarose powder. Determine the gel percentage needed according to manufacturer's instructions.

%	0.5%	1%	2%	3%	4%
Mass of agarose/g	0.5	1	2	3	4

- Pour in 100mL 1X TBE buffer with the powder into a clean bottle of appropriate volume.
- Put into a microwave and heat it for 3 – 5 minutes. Note: boiling of the solution is needed.
- Carefully wrap the bottle with paper towels. Let it cool on bench for around 30 seconds.
- Pour in into the gel caster and allow it to set (~15 minutes)
- After it is set, put in on the gel tank with the wells closer to the negative electrode.
- Pour in 1X TBE to cover the gel.
- Load samples.

Polyacrylamide Gel

%	30% Acrylamide (29:1)	ddH_2O	10 TBE	10% APS		TEMED

8%	3200 uL	7600 uL	1200 uL	200 uL		10 uL
12%	4800 uL	6000 uL				
15%	6000 uL	4800 uL				

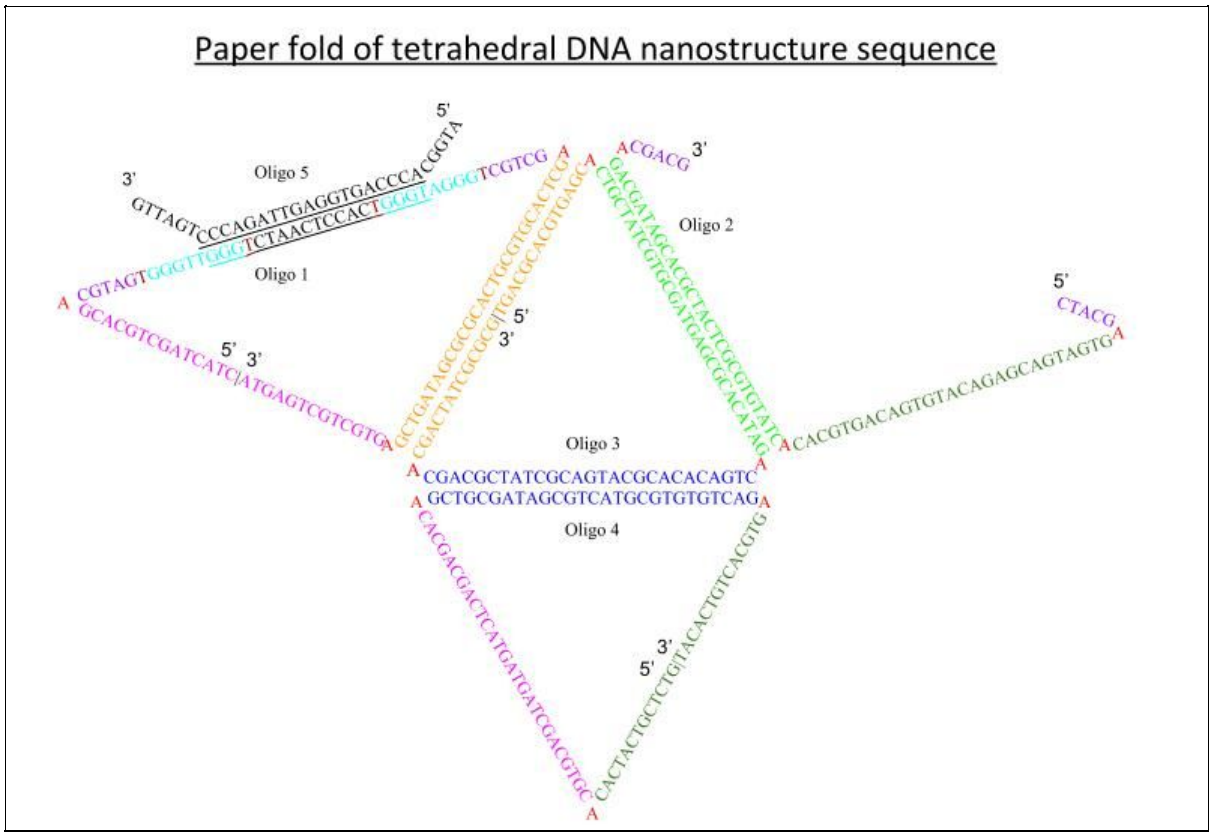
- Pipette into a clean 50mL Falcon tube the chemicals needed according to the percentage required, except TEMED
- Once the gel caster is ready, pipette 10uL TEMED into the mixture;
- Vortex quickly to mix;
- Pour it into the gel caster and quickly insert the comb;
- Let it polymerize (~15 minutes);
- Load samples;

DNA Nanostructure Assembly

Equal amounts of the oligos are mixed in TM buffer (20 mM Tris, 50mM $MgCl_2$, pH 8), making the final concentration of each oligo to be 10μM. The oligos are incubated at 95℃ for 5 minutes and cooled down to 25℃ with a drop of 0.5℃ every 30 seconds in a thermal cycler.

The following table shows the sequence of our tetrahedral DNA nanostructure. Cyan parts show the split G-quadruplex and the underlined sequences are the complementary sequence between O1 and O5. The colour code used is the same as that in the paper fold of the structure (below the table).

Oligo Name	Sequence (5' to 3')
O1 (97nt)	CTACTAGCTGCACGACGTAGTGGGTTGGGTCTAACTCCAC TGGGTAGGGTCGTCGAGCTCACGTGCGTCACGCGCGATAG TCGAGTGCTGCTGAGTA
O2 (67nt)	CTACGAGTGATGACGAGACATGTGACAGTGCACACTATGT GCGCTCATCGCACGATAGCAGACGACG
O3 (84nt)	TGACGCACGTGAGCACTGCTATCGTGCGATGAGCGCACAT AGACTGACACACGCATGACGCTATCGCAGCACGACTATCG CGCG
O4 (84nt)	GTCTCGTCATCACACGTGCAGCTAGTAGTACTCAGCAGCA CAGCTGCGATAGCGTCATGCGTGTGTCAGAGTGCACTGTC ACAT
O5 (30nt)	ATGGCACCCAGTGGAGTTAGACCCTGATTG



Gel Electrophoresis Analysis

The assembly of DNA nanostructure is analysed by 12% PAGE where the combinations of oligos (5µl, 10µM) are loaded. For analysis by 1% agarose gel, 10µl samples (10µM) are loaded. All the gels are run at a constant voltage of 100V. GelRed is used to prestained the gels.

For the analysis of strand displacement, equimolar (10µM final) DNA nanostructure and nucleic acid input are mixed and incubate at room temperature for 30 minutes in a shaker. The mixture (5µl, 10µM) is then loaded to 12% polyacrylamide gel. The gel is run at a constant voltage of 100V. GelRed is used to prestained the gel.

ABTS Assay

ABTS assay is used to detect G-quadruplex. DNA nanostructure (100nM final), nucleic acid input (100nM final) and hemin (400nM) are added to 23µl buffer (50 mM Tris–HCl, 150 mM NH4Cl, 20 mM KCl, and 0.03% Triton X-100, pH 7.5). The mixture is incubated at room temperature for 30 minutes in a shaker. 100µl 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) solution (from Roche CAT ELISA Kit) and 15µl H_2O_2 (12mM final) are added to the mixture, making the final volume to be 150µl. The reaction mixture is transferred to a 96-well plate and absorbance at 420nm is measured with a microplate spectrophotometer.

Preparation of DNA working solutions

From <u>STOCK solution (100 M)</u> to <u>WORKING solution (1 M)</u>

Buffer used: TM buffer (40 mM Tris, 100mM $MgCl_2$, pH 8)

Preparation of TM buffer (volume: 50ml)

1. Dilute 1M Tris solution into 40mM by adding 2ml 1M Tris solution to 48ml ddH2O.
2. Dissolve 1.0165g $MgCl_2 \cdot 6H2O$ into the solution.
3. Adjust pH to 8 by adding 1M HCl drop by drop.

Procedures

1. Wipe the bench and gloves with 70% ethanol.
2. Briefly vortex the stock solutions before dilution.
3. Prepare the followings.

Eppendorf/ PCR tube (label as)	Volume of 1X TM buffer*	Volume of 100µM stock solution	Final volume	Final conc.
O1 (5µM)	95µL	5µL	100µL	5µM
O2 (5µM)	95µL	5µL	100µL	5µM
O3 (5µM)	95µL	5µL	100µL	5µM
O4 (5µM)	95µL	5µL	100µL	5µM
O5 (5µM)	95µL	5µL	100µL	5µM
O1 (25µM)	16.5µL	5.5µL	22µL	25µM
O2 (25µM)	16.5µL	5.5µL	22µL	25µM
O3 (25µM)	16.5µL	5.5µL	22µL	25µM
O4 (25µM)	16.5µL	5.5µL	22µL	25µM
O5 (25µM)	16.5µL	5.5µL	22µL	25µM
Input (5µM)	95µL	5µL	100µL	5µM

4. Mix well by tapping and then briefly centrifuge the eppendorfs.
5. Store the working solution at -20°C

Tetrahedron assembly and preparation of DNA solutions for PAGE

1. Tap the DNA solutions and centrifuge them.
2. Prepare the followings.

PCR tube (label as)	**1X** TM buffer	O1 (5μM)	O2 (5μM)	O3 (5μM)	O4 (5μM)	O5 (5μM)	Final volume	Final conc.
A1	6μL	2μL	2μL	/	/	/	10μL	1μM
A2		2μL	/	2μL	/	/		
A3		2μL	/	/	2μL	/		
A4		2μL	/	/	/	2μL		

PCR tube (label as)	**1X** TM buffer	O1 (5μM)	O2 (5μM)	O3 (5μM)	O4 (5μM)	O5 (5μM)	Final volume	Final conc.
A5	6μL	/	2μL	2μL	/	/	10μL	1μM
A6		/	2μL	/	2μL	/		
A7		/	2μL	/	/	2μL		
A8		/	/	2μL	2μL	/		
A9		/	/	2μL	/	2μL		
A10		/	/	/	2μL	2μL		

PCR tube (label as)	**1X** TM buffer	O1 (5μM)	O2 (5μM)	O3 (5μM)	O4 (5μM)	O5 (5μM)	Final volume	Final conc.
B1	4μL	2μL	2μL	2μL	/	/	10μL	1μM
B2		2μL	2μL	/	2μL	/		
B3		2μL	2μL	/	/	2μL		
B4		2μL	/	2μL	2μL	/		
B5		2μL	/	2μL	/	2μL		
B6		2μL	/	/	2μL	2μL		

PCR tube (label as)	**1X** TM buffer	O1 (5μM)	O2 (5μM)	O3 (5μM)	O4 (5μM)	O5 (5μM)	Final volume	Final conc.
B7	4μL	/	2μL	2μL	2μL	/	10μL	1μM

B8		/	2μL	2μL	/	2μL		
B9		/	2μL	/	2μL	2μL		
B10		/	/	2μL	2μL	2μL		

PCR tube (label as)	**1X** TM buffer	O1 (5μM)	O2 (5μM)	O3 (5μM)	O4 (5μM)	O5 (5μM)	Final volume	Final conc.
C1	2μL	2μL	2μL	2μL	2μL	/	10μL	1μM
C2		2μL	2μL	2μL	/	2μL		
C3		2μL	2μL	/	2μL	2μL		
C4		2μL	/	2μL	2μL	2μL		
C5		/	2μL	2μL	2μL	2μL		
Output	6μL	2μL O5(5μM) + 2μL Input (5μM)						

PCR tube (label as)	1X TM buffer	O1 (25μM)	O2 (25μM)	O3 (25μM)	O4 (25μM)	O5 (25μM)	Final volume	Final conc.
Tetra	/	10μL	10μL	10μL	10μL	10μL	50μL	5μM

3. Tap and centrifuge the PCR tubes.
4. Incubate the PCR tubes at 95°C for 5 min and cool down to 25°C with 0.5°C drop every 30 seconds using a thermal cycler.

Strand Displacement (for PAGE and agarose)

1. Dilution of tetra (5μM). Label it as Td.

ddH2O	Tetra (5μM)	Input (5μM)	Final volume	Final conc.
12μL	4μL	4μL	20μL	1μM

2. Place the tubes in a shaker and incubate them at room temperature for 30 minutes for strand displacement.

Native Page

1. Prepare four **12%** polyacrylamide gels using the formula below.

Gel %	30% Acrylamide (29:1)	ddH2O	10X TBE	10% APS	TEMED
12%	4.8mL	6mL	1.2mL	200µl	10µl

2. Dilution of Tetra XO5 and label it as C1.

ddH2O	Tetra XO5 (5µM)	Final volume	Final conc.
8µL	2µL	10µL	1µM

3. Dilution of Tetra and label it as Tetra (1µM).
   (Half volume for PAGE, half volume for agarose)

ddH2O	Tetra (5µM)	Final volume	Final conc.
16µL	4µL	20µL	1µM

4. Add 1µL of loading buffer to 10µL of 1µM DNA solutions.
5. Load the followings. (DNA ladder: 2µL, DNA sample: <8µL, 1X loading dye: <8µL)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A	Ladder	O1.6	O2	O3	O4	O5	Input	A1	A2	A3
B		A4	A5	A6	A7	A8	A9	A10	B1	B2
C		B3	B4	B5	B6	B7	B8	B9	B10	1X loading buffer
D		C1	C2	C3	C4	C5	Tetra	Tetra+ input	Output	

6. Run the gel for until the bands of dye reach ¾ of the length of the gel

Agarose gel electrophoresis (1%)

Procedures

1. Measure out 1g of agarose.
2. Pour agarose powder into microwavable flask along with 100mL of 1xTBE.
3. Microwave for 1-3min (until the agarose is completely dissolved and there is a nice rolling boil).
4. Let agarose solution cool down for 5min.
5. Pour the agarose into a gel tray with the well comb in place.

6. Let the newly poured gel sits at room temperature for 20-30 minutes, until it has completely solidified.
7. Fill gel box with 1xTBE until the gel is covered.
8. Add DNA loading dye to samples.
9. Load the samples into the wells.
10. Run the gel at 100V for 1 hour.

(The above protocol is adapted from Addgene Agarose Gel Electrophoresis Protocol, which can be found at https://www.addgene.org/plasmid-protocols/gel-electrophoresis/)

ABTS assay

Buffer used in the ABTS assay: 50 mM Tris–HCl, 150 mM NH_4Cl, 20 mM KCl, 0.03% Triton X-100 (pH 7.5)

Preparation of the buffer (volume: 100ml)

1. Dilute 1M Tris buffer into 50mM by adding 5ml 1M Tris buffer to 95ml ddH2O.
2. Dissolve 0.8g NH_4Cl and 0.15g KCl into the solution.
3. Adjust pH of the solution to 7.5 by adding 1M HCl drop by drop.
4. Add 30µl Triton X-100 to the solution and shake vigorously.

Procedures of ABTS assay

Reagent	**Concentration**	**Volume**	**Final concentration**
Tetrahedron	5µM	3µl	100nM
Input	5µM	3µl	100nM
Hemin	10µM	6µl	0.4µM
ABTS	(from CAT ELISA kit)	100µl	unknown
H_2O_2	120mM	15µl	12mM
Buffer	see above	23µl (make up the final volume to 150µl)	/
Total Volume		150µl	

1. Add 23µl buffer into an eppendorf.
2. Add 3µl tetrahedron (5µM), 3µl input (5µM) and 6µl hemin (10µM) into the above eppendorf. Briefly vortex the solution.

3. Incubate the eppendorf at room temperature for 30 minutes in a shaker.
4. Add 100µl ABTS solution (from Roche? CAT ELISA Kit) and 15µl 120mM H_2O_2 into the eppendorf. Briefly vortex the solution.
5. Transfer the solution (final volume: 150µl) to a 96-well plate.
6. Measure the absorbance at 420nm using a spectrophotometer.
7. Prepare appropriate controls by adjusting the volume of buffer used so that the final volume is 150µl. (For example, for a control without the addition of 3µl input, add 26µl buffer in step 1.)

RNA target detection…

LOD...

Inserts preparation

Digestion 1 (O1 & O5, and O1, O2, O3, O4 & O5)

	Tube label	**CutSmart® (10X)**	**SpeI**	**XbaI**	**DNA**	**ddH_2O**
p_O15	p_15 O1 xSpeI	1uL	1uL	---	O1 G-Block 4 uL	4uL
	p_15 O5 xXbaI		---	1uL	O5 G-Block 4 uL	
Total: 10uL						
p_O12345	p_1-5 O1 xSpeI	1uL	1uL	---	O1 G-Block 4 uL	4uL
	p_1-5 O2 xXbaI		---	1uL	O2 G-Block 4 uL	
	p_1-5 O3 xSpeI		1uL	---	O3 G-Block 4 uL	
	p_1-5 O4 xXbaI		---	1uL	O4 G-Block 4 uL	
	Total: 10uL					
	p_1-5 O5 xXbaI	1uL	---	1uL	O5 G-Block 4 uL	4uL

- Incubate at 37°C, 1 hour
- Heat kill at 80°C, 20 minutes

Ligation 1

	Tube label	**T4 Ligase Buffer (10X)**	**T4 DNA ligase**	**DNA**				**ddH_2O**
				1		2		
p_O15	O15	2 uL	1 uL	p_15 O1 xSpeI	7 uL	p_15 O5 xXbaI	7 uL	
Total: 17uL								
p_O12345	O1+O2	2 uL	1uL	p_1-5 O1 xSpeI	7 uL	p_1-5 O2 xXbaI	7 uL	
	O3+O4			p_1-5 O3 xSpeI	7 uL	p_1-5 O4 xXbaI	7 uL	

- Incubate at room temperature, 10 minutes;
- Heat kill at 80°C, 20 minutes
- Chill **on ice**;

Remaining p_15 or p_1-5 solutions are needed for gel electrophoresis. Do NOT throw them away.

Digestion 2 (O1+O2 & O3+O4)

	Tube label	**CutSmart® (10X)**	**SpeI**	**XbaI**	**DNA**		**ddH$_2$O**
p_O12345	p_1-5 O1+O2 xSpeI	1.5uL	1uL	---	O1+O2	12 uL	0.5uL
	p_1-5 O3+O4 xXbaI		---	1uL	O3+O4		
Total: 15 uL							

- Incubate at 37°C, 1 hour
- Heat kill at 80°C, 20 minutes

Ligation 2

	Tube label	**T4 Ligase Buffer (10X)**	**T4 DNA ligase**	**DNA**				**ddH$_2$O**
				1		2		
p_O12345	O1-O4	3 uL	1.5 uL	p_1-5 O1+O2 xSpeI	11 uL	p_1-5 O3+O4 xXbaI	11 uL	
Total: 26.5 uL								

- Incubate at room temperature, 10 minutes;
- Heat kill at 80°C, 20 minutes
- Chill **on ice**;

Digestion 3 (O1-O4)

	Tube label	**CutSmart® (10X)**	**SpeI**	**XbaI**	**DNA**		**ddH$_2$O**
p_O12345	p_1-5 O1-O4 xSpeI	2.3uL	1uL	---	O1-O4	20 uL	---
Total: 23.3 uL							

- Incubate at 37°C, 1 hour
- Heat kill at 80°C, 20 minutes

Ligation 3

	Tube label	**T4 Ligase Buffer (10X)**	**T4 DNA ligase**	**DNA**				**ddH$_2$O**
				1		2		
p_O12345	O12345	2.5 uL	1.5 uL	p_1-5 O1-O4 xSpeI	17 uL	p_1-5 O5 xXbaI	1 uL	---
Total: 22.5 uL								

- Incubate at room temperature, 10 minutes;
- Heat kill at 80°C, 20 minutes
- Chill **on ice**;
- **Remaining p_15 or p_1-5 solutions are needed for gel electrophoresis. Do NOT throw them away.**

Plasmid preparation - Featured parts

- Digest G-block fragments and linearised p_SB1C3 backbone with pstI and EcoRI under buffer 3.1 following NEB optimized RE digestion protocol.
- Incubate at 37°C, 1 hour
- Heat kill at 80°C, 20 minutes
- Ligate with T4 ligation following NEB ligation protocol, try backbone:inseert ratios of 1:1, 1:3 and 1:10;
- Incubate at room temperature, 1 hour
- Heat kill at 80°C, 20 minutes
- Transform half to whole of the ligated mixture, following NEB high efficiency (DH-10B) transformation protocol;
- After the recovery stage (1 hour 37°C incubation), spin down and remove supernatant until around 200uL left;
- Resuspend pellet;
- Spread all on to selection plates and incubate overnight at 37°C;

Plasmid preparation - Featured parts

Digestion of inserts and backbone (O12345 and O15)

	Tube label	**CutSmart® (10X)**	**EcoRI**	**PstI**	**DNA**		**ddH_2O**
p_O12345	O12345 x EcoRI x PstI	2uL	1uL	1uL	O12345	15 uL	1uL
Total: 20 uL							
p_O15	O15 x EcoR1 x PstI	2uL	1uL	1uL	O15	13 uL	3uL
Total: 15.5 uL							
p_SB1C3	1C3 x EcoRI x PstI	1uL	1uL	1uL	p_SB1C3	6 uL	1uL
Total: 10 uL							

- Incubate at 37°C, 1 hour
- Heat kill at 80°C, 20 minutes

Ligation (making p_O12345 and p_O15)

	Tube label	**T4 Ligase Buffer (10X)**	**T4 DNA ligase**	**DNA**				**ddH_2O**
				1		2		
p_O15	p_O15	2 uL	1 uL	1C3		p O15		

				x EcoRI x PstI		x EcoR1 x PstI		
p_O12345	p_O12345	2 uL	1uL	1C3 x EcoRI x PstI		O12345 x EcoRI x PstI		

- Incubate at room temperature, 10 minutes;
- Heat kill at 80°C, 20 minutes
- Chill p_O15 and p_O12345 **on ice** until ready for transformation into 100uL of DH10-beta E. Coli

Calculation needed.

PART 3. AGAROSE GEL ELECTROPHORESIS

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10kb ladder														

Transformation

Part 1: High Efficiency Transformation Protocol using NEB 10-beta Competent E. coli (High Efficiency) (C3019) from NEB website

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1. C3019I: Thaw a tube of NEB 10-beta Competent E. coli cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 100 µl of cells into a transformation tube on ice.

2. Add 5 µl of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.

3. Place the mixture on ice for 30 minutes. Do not mix.

4. Heat shock at exactly 42°C for exactly 60 seconds. Do not mix.

5. Place on ice for 5 minutes. Do not mix.

6. Pipette 950 µl of room temperature SOC into the mixture.

7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.

8. Warm selection plates to 37°C.

9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.

10. Spread 50-100 µl of each dilution onto a selection plate and incubate overnight at 37°C.

Part 2: QIAprep® Spin Miniprep Kit
(Adapted for our designs, original protocol at https://www.qiagen.com/us/resources/download.aspx?id=56b0162c-23b0-473c-9229-12e8b5c8d590&lang=en.)

1. Pellet 1–5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 x g) for 3min at room temperature (15–25°C).

2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.

3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.

4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.

5. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.

6. Apply 800 µl supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. Centrifuge for 30–60 s and discard the flow-through

7. Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE. Centrifuge for 30–60 s and discard the flow-through. Transfer the QIAprep 2.0 spin column to the collection tube.

8. Centrifuge for 1 min to remove residual wash buffer.

9. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl water to the center of the QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.